Scientific Note

Culex pipiens-quinquefasciatus hybrids identified in West Texas

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The *Culex pipiens* complex (Diptera: Culicidae) has historically challenged mosquito taxonomists due to the scarcity of morphological differences between different forms or even distinctive species that otherwise differ in multiple factors such as host selection, mating behavior, oviposition site selection, larval habitat, and resistance to cold weather (Vinogradova 2000, Fonseca et al. 2004, Bahnck and Fonseca 2006, Harbach 2012). In North America, the complex is currently known to contain two distinct species; *Culex pipiens* (Linnaeus) and *Culex quinquefasciatus* (Say). Both species hybridize in areas where populations overlap and their distribution areas include a distinctive hybrid zone between populations of *Cx. pipiens* in the north and *Cx. quinquefasciatus* in the south (Vinogradova 2000, Smith and Fonseca 2004, Harbach 2012). This hybrid zone traditionally includes the area between latitudes 36°-39° N (Barr 1957), while later studies claim it most likely stretches further to the north and south (Edillo et al. 2009, Kothera et al. 2009). However, empirical data in support of this is usually restricted to Culex populations east of the Mississippi river or west of the Sierra Nevada (Smith and Fonseca 2004, Kothera et al. 2009, Edillo et al. 2009). Data about the hybrid zone outside these areas, particularly in Texas, are very scarce. Although Barr (1957) has reported finding hybrids in Lubbock, TX (latitude 33° 5' N), we could not find any additional studies reporting this. Hybrids are not mentioned in mosquito surveys (Bradford et al. 2008) or databases (Hellmann et al. 2013) where populations of northwest Texas are referred to as *C. quinquefasciatus*. The species *Culex pipiens* contains two forms (or subspecies) in North America; *Cx. pipiens* f. pipiens and *Cx. pipiens* f. molestus (known in the past as *Culex molestus* Forskål). These forms hybridize with each other and with *C. quinquefasciatus* when their distributions overlap, creating three types of hybrids (Smith and Fonseca 2004, Bahnck and Fonseca 2006).

We examined the identity of *Cx. pipiens* in Lubbock, TX, as part of an experiment conducted on larvae life history. Traditional methods distinguish between the two species and their hybrids according to the ratio of the ventral and dorsal arms composing the male genitalia (reviewed in Barr 1957, Harbach 2012). Morphological differences between the two *Cx. pipiens* forms (and their hybrids) vary within populations and are often described as indistinguishable (Vinogradova 2000, Bahnck and Fonseca 2006, Harbach 2012). We used the polymerase chain reaction PCR-based assays described by Smith and Fonseca (2004) and Bahnck and Fonseca (2006) that provide rapid and reliable identification between the two forms of *Cx. pipiens*, *Cx. quinquefasciatus,* and the hybrids of all three. Egg rafts were collected from oviposition traps (cattle tanks and buckets) located at four sites in Lubbock during October, 2012. Larvae were reared to the 4th instar and seven out of 15 were morphologically identified as *Cx. pipiens* (Darsie and Ward 2005). Larvae were placed in 75% ethanol prior to DNA analysis. Other species found were *Culex tarsalis* (Coquillett), *Culex restuans* (Theobald), and *Culiseta inornata* (Williston).

DNA was isolated and purified from larvae using the Roche High Pure PCR template kit. We used a combination of ACEquin and ACEpip with the reverse primer B1246s to differentiate between species (Smith and Fonseca 2004) and the forward primer CQ11F2 with the reverse pipCQ11R and molCQ11R to distinguish between the three (Bahnck and Fonseca 2006). Briefly, ACEpip anneals to both *Cx. pipiens* forms while ACEquin anneals to *Cx. quinquefasciatus*; therefore, species identification is based on the presence or absence of a PCR amplicon and *pipiens-quinquefasciatus* hybrids are identified as having two PCR amplicons (sizes 610 pb: ACEpip- B1246s and 274 bp: ACEquin-B1246s; Smith and Fonseca 2004). The primer molQC11R anneals to both *C. quinquefasciatus* and *Cx. pipiens* f. *molestus*, but not to *C. pipiens* f. *pipiens*, whereas pipQC11R anneals only to *Cx. pipiens* f. *pipiens*. Paired with the forward primer QC11F2, these primers should form ~250 bp band for *Cx. pipiens* f. *molestus* and *Cx. quinquefasciatus*, while the band for *Cx. pipiens* f. *pipiens* is expected to be about 70 bp smaller (Bahnck and Fonseca 2006). The combination of all four primer pairs should thus distinguish among these species, forms, and their hybrids (Bahnck and Fonseca 2006). PCR assays were amplified using a 55° C annealing temperature, electrophoresed on 1.5% agarose gel, and visualized using ethidium bromide staining. A water negative control was run with each primer pair.

All four pairs of six primers annealed to DNA from all seven individuals identified. All seven showed the same bands as the individual presented in Figure 1. This result identified them as *Cx. quinquefasciatus- Cx. pipiens* f. *pipiens* hybrids, although the presence of *Cx. pipiens* f. *molestus* ancestry (a three-way hybrid) cannot be completely rejected solely by these results (Bahnck and Fonseca 2006).

Understanding population dynamics is a critical step in controlling pest populations. The borders of the *Cx. pipiens-quinquefasciatus* hybrid zone differ between the measuring techniques and require the presence of a certain percentage of hybrids within the population (Kothera et al. 2009). Barr (1957) found two hybrids from a sample of 42 individuals. Based on these data, it was concluded that Lubbock is well to the south of the hybrid zone, although noting that the percentage of hybrids in the population is most likely much larger. Although our sample size was relatively small, the fact that all seven individuals tested were actually hybrids indicates a much higher percentage of hybridization than reported, and thus to the possibility that the

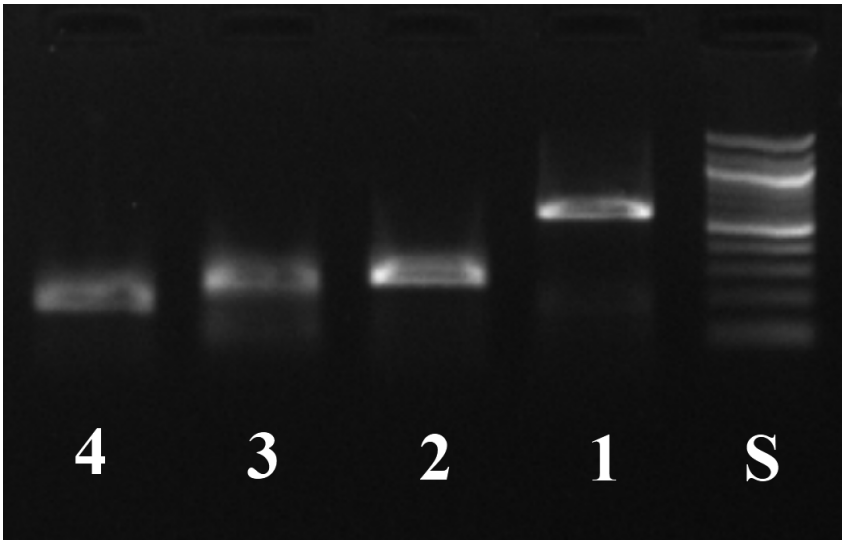

Figure 1. PCR products of the four primer pairs for a single individual from the sample run on a 1.5% agarose gel. Reverse primer B1246s coupled with forwards (1) ACEpip and (2) ACEquin. Forward primer CQ11F2 coupled with reverse (3) molCQ11R and (4) pipCQ11R. Products were run with a standard (S) 100 bp ladder (Bioscience Inc.).

hybrid zone stretches further south than traditionally defined (as suggested by Kothera et al. 2009). More information is needed to define the actual borders of this zone, especially in areas that were overlooked in previous studies for applied reasons and population studies. The two species of the pipiens complex are considered as important vectors for several pathogens, including the West Nile virus (Turell et al. 2001), for which the number of human infections is constantly increasing in northwest Texas (Nolan et al. 2013). Kothera et al. (2009) points to possible differences in efficiency between hybrids and parental species as WNV vectors, further emphasizing the importance of knowledge about the actual borders of the hybrid zone in that region.

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